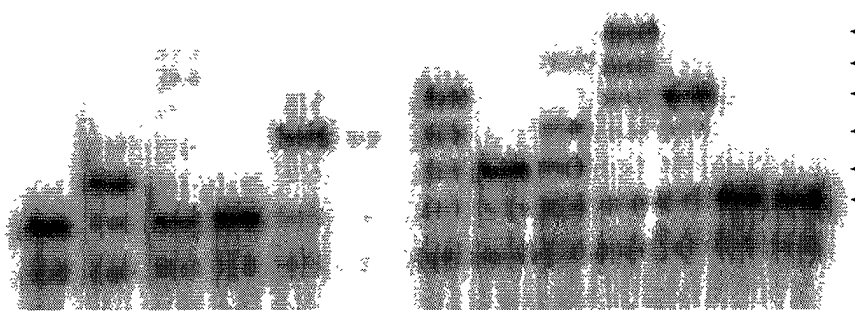
T0E02T' T29L00D7E

Fig.1

Incorporate: GATC AG AAAG
(5' to 3')



γ implies presence of an ANS-tag attached via the dNTP γ phosphate

Document Translation

Fig.2

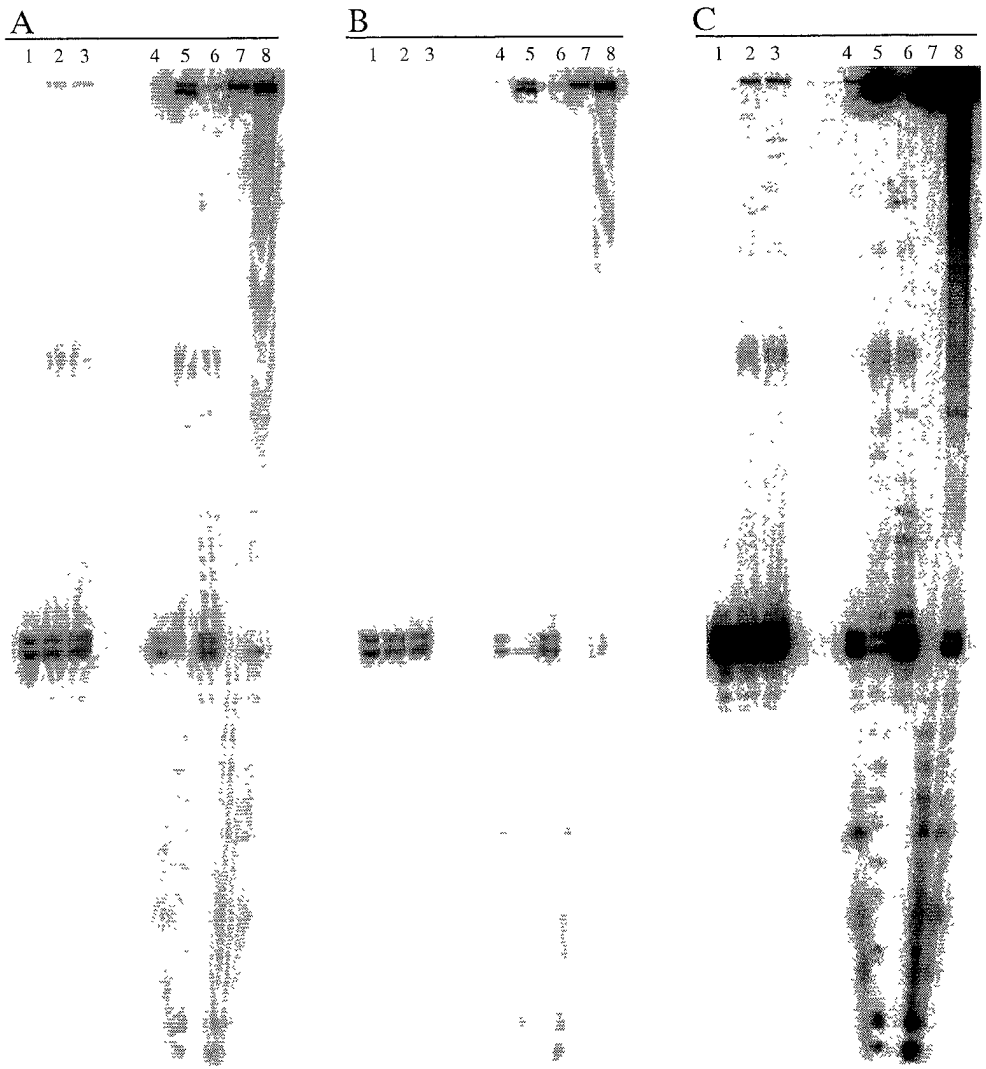


Fig.3

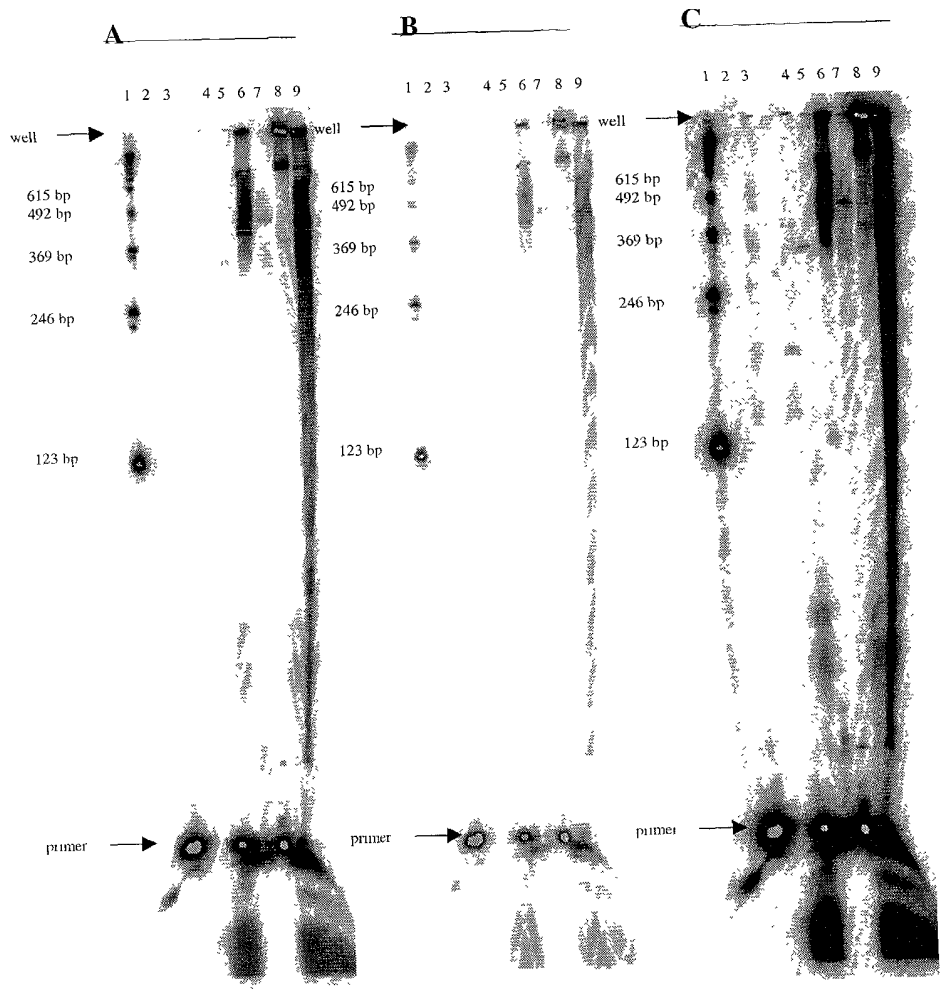
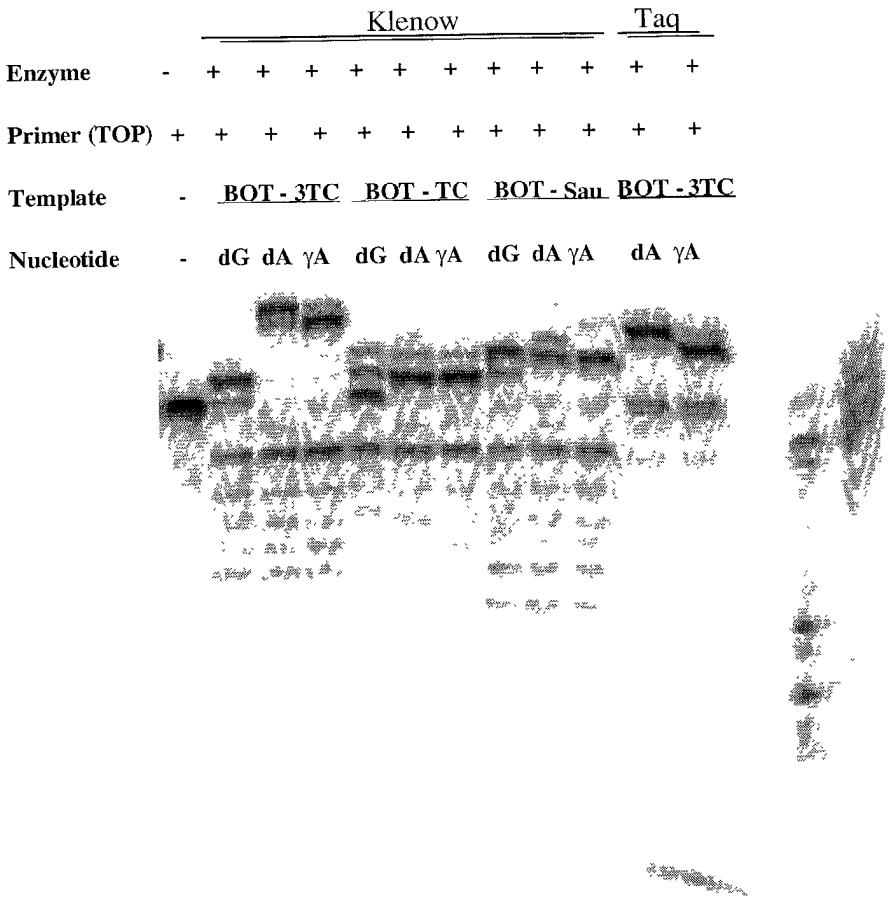
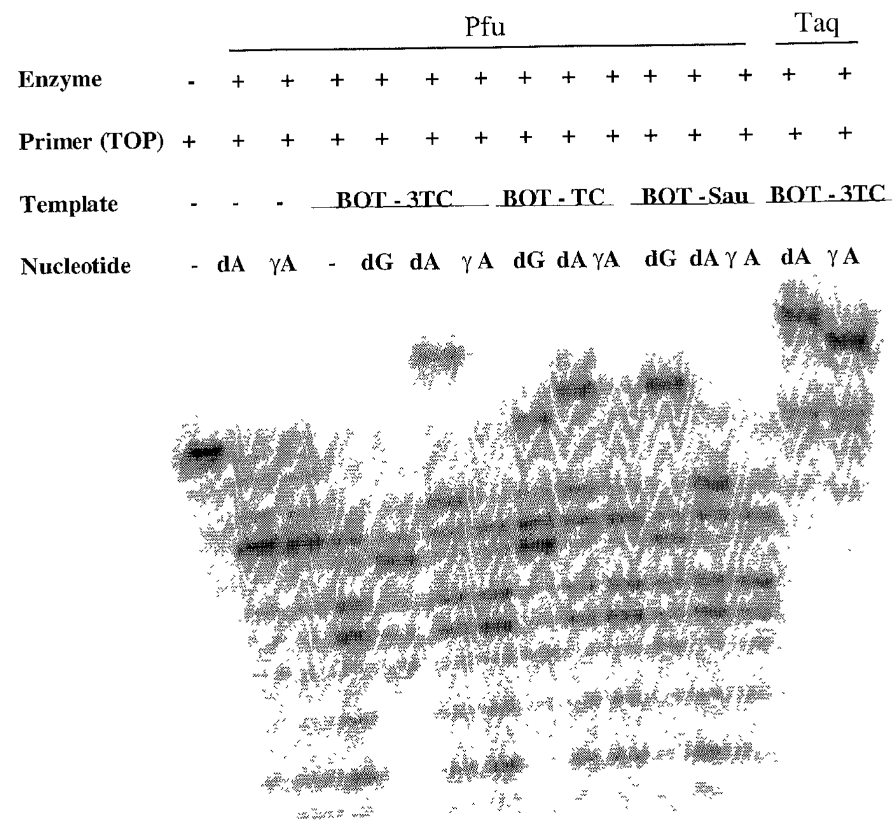


Fig.4



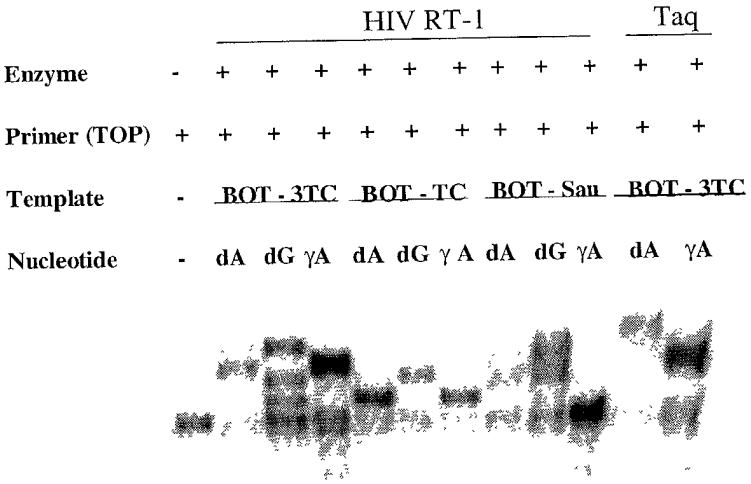
γ implies presence of an ANS-tag attached via the dNTP γ phosphate

Fig.5



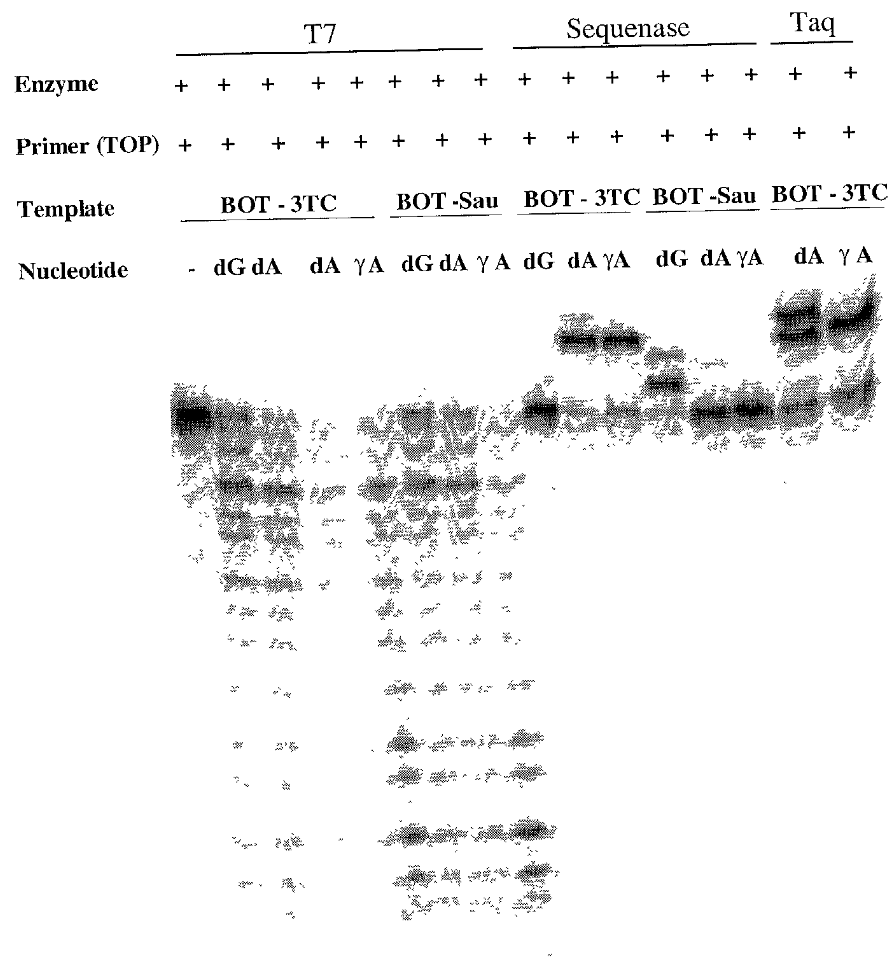
γ implies presence of an ANS-tag attached via the dNTP γ phosphate

Fig.6



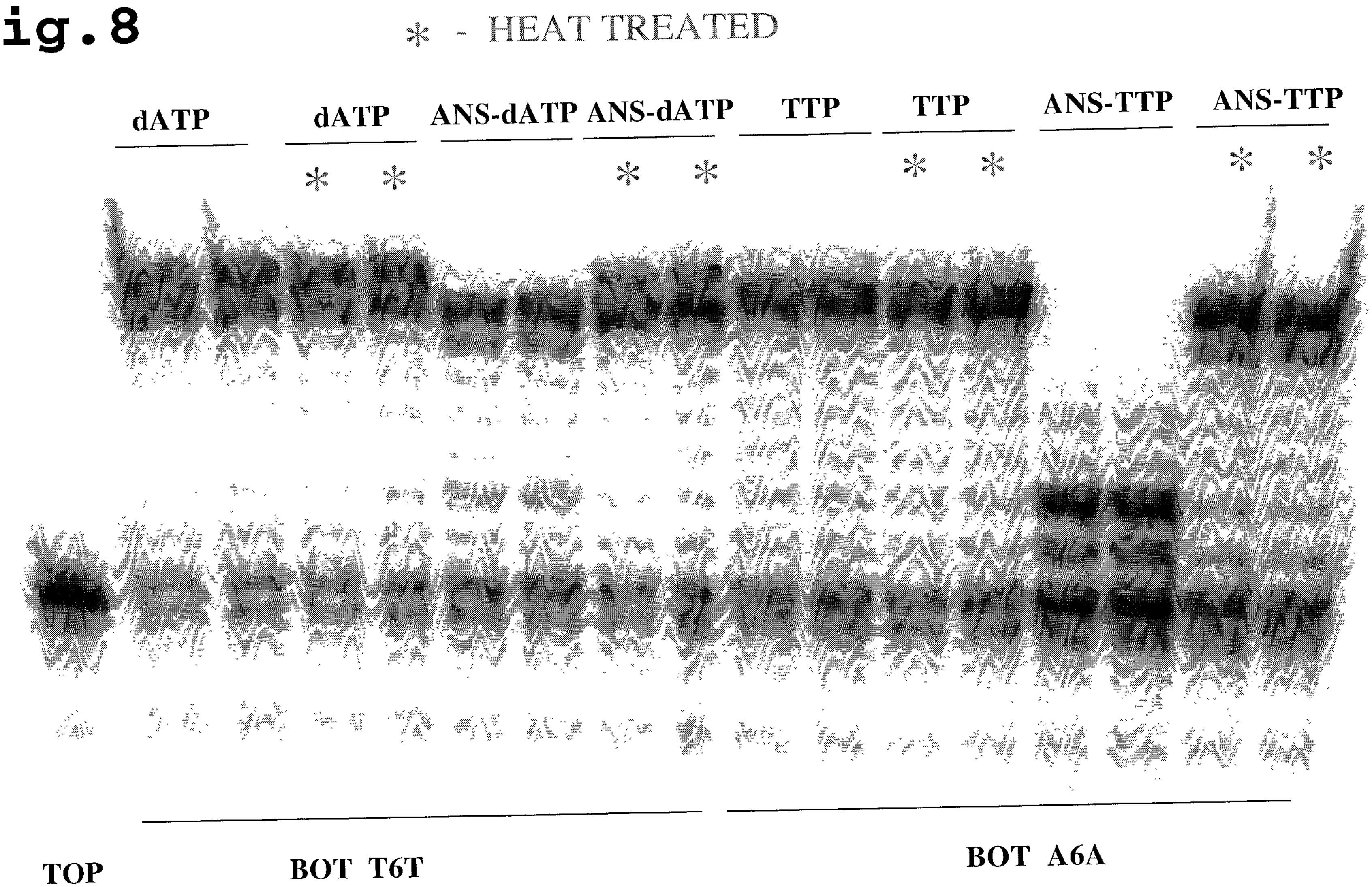
γ implies presence of an ANS-tag attached via the dNTP γ phosphate

Fig.7



γ implies presence of an ANS-tag attached via the dNTP γ phosphate

Fig.8

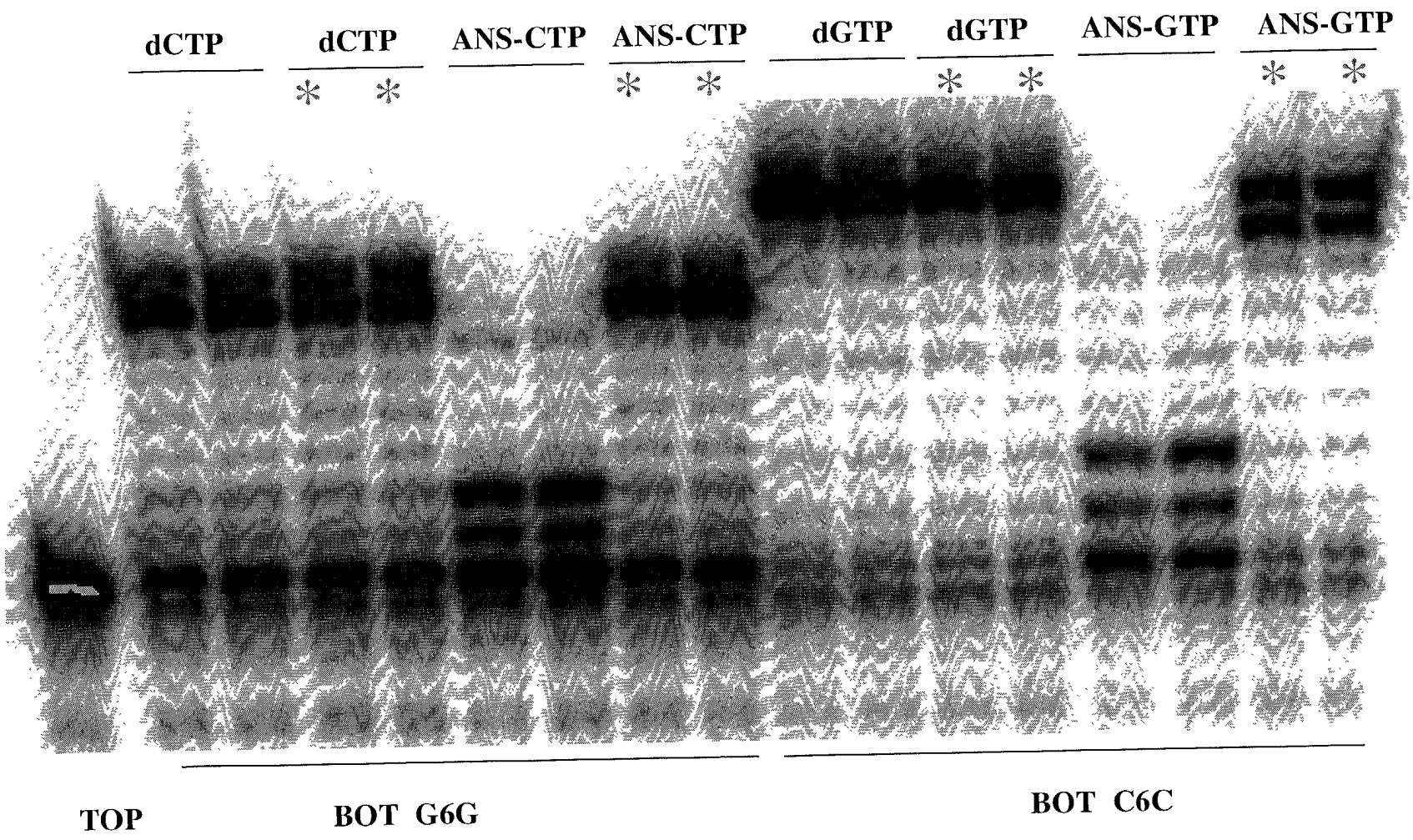


10μM each dNTP; *Taq DNA Polymerase; extension 30' @ 37°C*

TOP SECRET TESTCODE

Fig.9

* - HEAT TREATED



10μM each dNTP; *Taq DNA Polymerase; extension 30' @ 37°C*

TOEDIT TEXTOOT

Fig.10

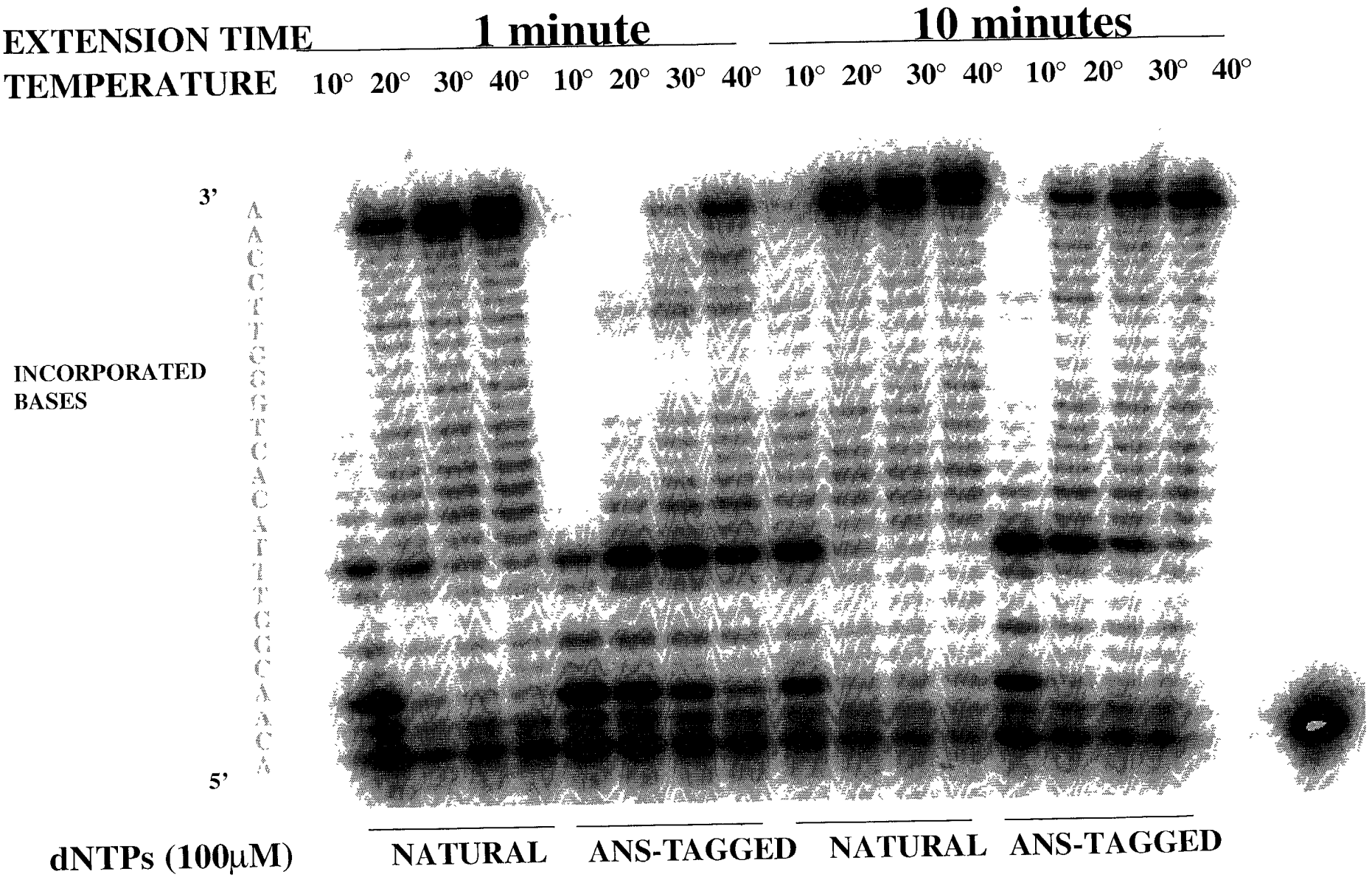
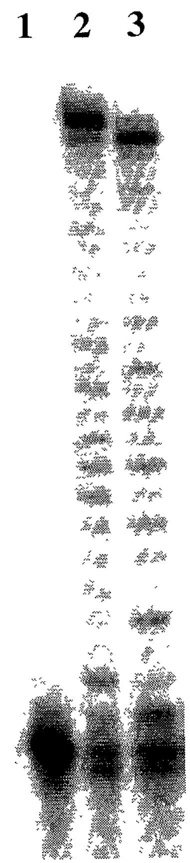


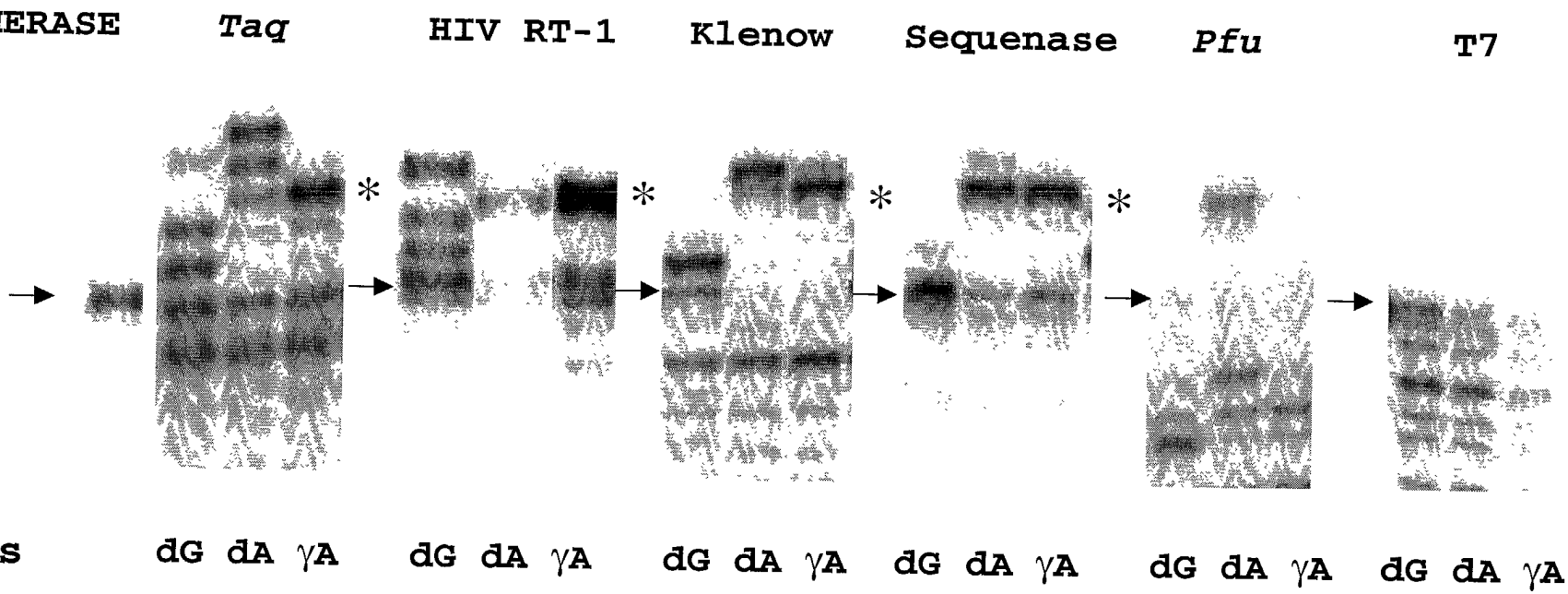
Fig.11

Primer sequence: 5' GGTACTAAGCGGCCGCATG 3'

Template sequence: 3' CCATGATTCGCCGGCGTAC<u>TGTTGCCAAATGTGACCCAAGGTT 5'</u>



Primer sequence: 5' GGTACTAAGCGGCCGCATG 3'
Template sequence: 3' CCATGATTCGCCGGCGTACTTTC5'



Different Polymerases React Differently to the ANS-γ-modified Nucleotides: primer extension reactions were performed to determine the ability of various polymerases to incorporate γ-tagged dNTPs during DNA polymerization.Control reactions contained natural dNTPs to monitor for template-directed nucleotide incorporation as well as for misincorporation. The reactions were performed in the appropriate buffer and contained the specified polymerase, primer/template duplex (radiolabeled 'TOP'primer annealed to 'BOT-3TC'template), and only the indicated dNTP. The reactions were carried out at room temperature or at 37°C for 30 minutes and were stopped by the addition of 0.5mM EDTA. The volume of the reaction was then reduced to approximately 2-4μl, loading dye was added and the polymerization products were electrophoresed through a 20% denaturing polyacrylamide gel.Arrows indicate the position of the free labeled 'TOP'. Asterisks indicate 3-base extension.

FIG. 12

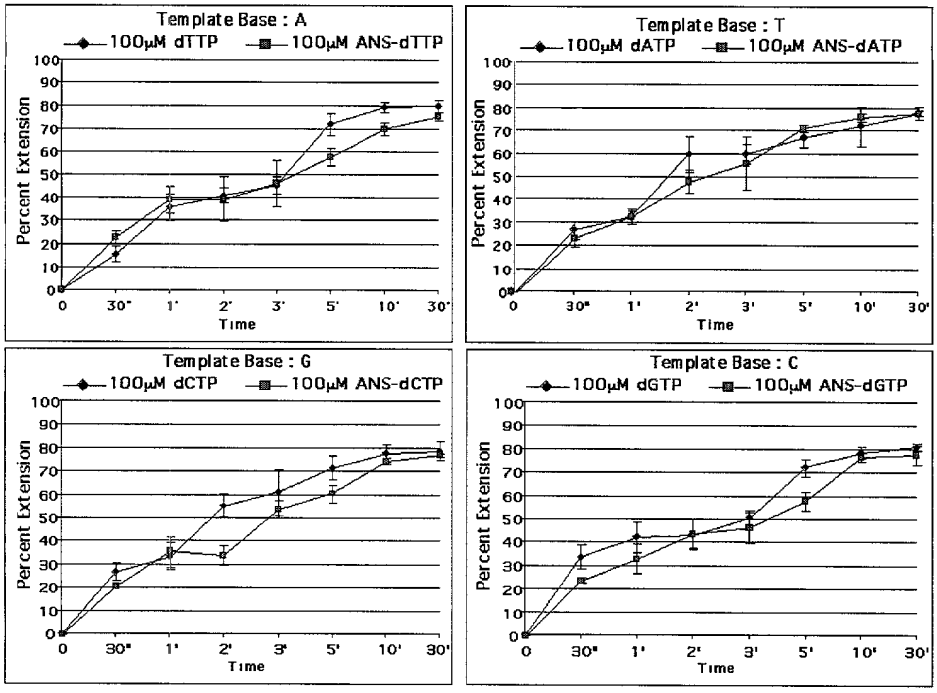
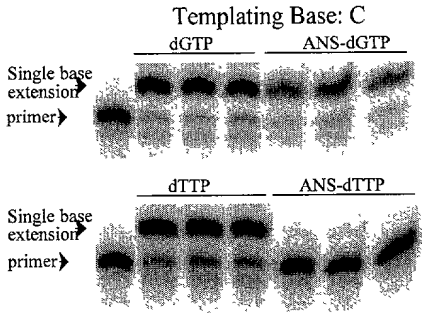

Template Base : A
100μM dTTP
100μM ANS-dTTP
Percent Extension
Time
Template Base : T
100μM dATP
100μM ANS-dATP
Percent Extension
Time
Template Base : G
100μM dCTP
100μM ANS-dCTP
Percent Extension
Time
Template Base : C
100μM dGTP
100μM ANS-dGTP
Percent Extension
Time

FIG. 13


Templating Base: C
dGTP
ANS-dGTP
Single base extension
primer
dTTP
ANS-dTTP
Single base extension
primer

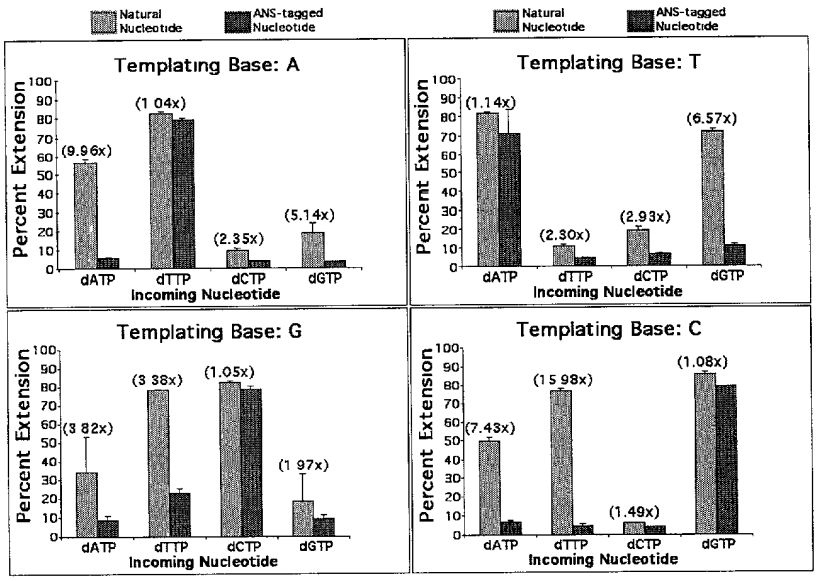

Natural Nucleotide
ANS-tagged Nucleotide
Templating Base: A
Percent Extension
(9.96x)
(1.04x)
(2.35x)
(5.14x)
dATP
dTTP
dCTP
dGTP
Incoming Nucleotide
Templating Base: T
(1.14x)
(2.30x)
(2.93x)
(6.57x)
Templating Base: G
(3.82x)
(3.38x)
(1.05x)
(1.97x)
Templating Base: C
(7.43x)
(15.98x)
(1.49x)
(1.08x)

FIG. 14